Weed Biology and Competition =



Relationship between Temperature and Heat Duration on Large Crabgrass (*Digitaria sanguinalis*), Virginia Buttonweed (*Diodia virginiana*), and Cock's-Comb Kyllinga (*Kyllinga squamulata*) Seed Mortality

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Thermal heat has been utilized for nonselective weed control methods. These methods are highly variable in application and efficacy. One effective weed–seed-control determining factor is achieving the thermal death point of targeted weed seeds. The thermal death point varies by weed species, temperature, and exposure time. Our objective was to determine the thermal death point of large crabgrass, cock's-comb kyllinga, and Virginia buttonweed at short thermal exposure periods. Studies conducted utilized 5 and 20 s exposure periods for incremental range, 60 to 250 C temperatures. Sigmoid regression curves were used to predict weed seed mortality by temperature and exposure time. A significant interaction between exposure period and temperature occurred for each weed species. Weed species increased in susceptibility to 20 s thermal heat as follows: Virginia buttonweed < cock's-comb kyllinga < large crabgrass and by 10 C for cock's-comb kyllinga. Virginia buttonweed achieved 50% mortality at 99 C for 5 and 20 s exposure periods, respectively, for these weed species.

Nomenclature: Large crabgrass, Digitaria sanguinalis (L.) Scop DIGSA; Virginia buttonweed, Diodia virginiana L. DIQVI; cock's-comb kyllinga, Kyllinga squamulata Thonn. ex Vahl, KYSQ.

Key words: Thermal seed death, thermal weed control, weed seed mortality.

El calor termal ha sido utilizado en métodos de control no-selectivo de malezas. Estos métodos son altamente variables en aplicación y eficacia. Un factor determinante del control de semillas de malezas es el poder alcanzar el punto de muerte termal de las semillas de las malezas objetivo. El punto de muerte termal varía según la especie de malezas, la temperatura y el tiempo de exposición. Nuestro objetivo fue determinar el punto de muerte termal de *Digitaria sanguinalis, Kyllinga squamulata* y *Diodia virginiana* bajo períodos cortos de exposición termal. Los estudios realizados utilizaron períodos de exposición de 5 y 20 s en un rango incremental de temperatura de 60 a 250 C. Curvas de regresión sigmoide fueron usadas para predecir la mortalidad de las semillas de las malezas según la temperatura y el tiempo de exposición. Una interacción significativa ocurrió entre el tiempo de exposición y la temperatura para cada especie. Las especies de malezas incrementaron en susceptibilidad a 20 s de calor termal como se describe a continuación: *D. virginiana* < *K. squamulata* < *D. sanguinalis.* Al incrementarse la exposición termal de 5 a 20 s se redujo la temperatura termal en 21 C para alcanzar 50% de mortalidad de *D. sanguinalis* y en 10 C para *K. squamulata. D. virginiana* alcanzó 50% de mortalidad a 99 C en períodos de exposición de 5 y 20 s. Estos datos indican que al menos 50% de la mortalidad de las semillas de malezas puede ser alcanzada a 99 y 103 C para períodos de exposición de 20 y 5 s, respectivamente, para estas especies de malezas.

Thermal weed control methods generate heat to kill weed seeds and emerged weeds (Bond et al. 2007). Techniques include soil solarization (Horowitz et al. 1983), flame weeding (Ascard 1995), infrared radiation (Ascard 1998), steaming and hot water (Anonymous 1999; Barberi et al. 2009; Trotter 1991), direct heat (Ascard et al. 2007; Hopkins 1936), electrocution (Vigneault et al. 1990), microwave radiation (Ascard et al. 2007), electrostatic fields (Diprose et al. 1984), irradiation (Suss and Bachthaler 1968), lasers (Couch and Gangstad 1974; Heisel et al. 2002; Mathiassen et al. 2006), and ultraviolet light (Andreasen et al. 1999). Reaching the thermal weed seed death point determines the efficacy of thermal weed control methods. The threshold temperature required to prevent germination is dependent on weed species, seed moisture level, and treatment duration (Riemens 2003).

The ability to achieve the optimal temperature for the required duration varies with energy source. Laboratory soil steaming for 90 s required temperatures between 65 and 75 C to kill individual weed species, losing 1 C per 60 s after the heat source is removed (Melander and Jrgensen 2005). Mobile steaming units are detrimental to weed species, reaching soil temperatures of 70 to 100 C for 3 to 8 min (White et al. 2000a,b). Heat exposure by solarization might require greater than 65 C for extended duration at the seed surface (Standifer et al. 1984). Utilizing solarization soil temperatures might exceed the thermal seed threshold for only a short period per day (0 to 2 h); therefore, several days or weeks of application are needed to accumulate sufficient heat exposure (Horowitz and Taylorson 1983).

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Thermal weed control efficacy not only depends on the energy source but also can vary because of the temperature and duration requirement of targeted weed species to achieve the thermal death point. Temperature to achieve thermal weed seed death varies with species (Egley 1983, 1990; Linke 1994; Rubin and Benjamin 1984). Hopkins (1936) reported lethal temperature varied by weed species when exposed to 15 min of thermal heat. The lethal death temperature of conditioned wild oat (Avena fatua L.) seeds (50% relative humidity at 25 C) was 105 C, whereas the lethal death temperature of conditioned redroot pigweed (Amaranthus retroflexus L.) seeds was 85 C. Imbibed broadleaf dock (Rumex obtusifolius L.) seeds exposed to 204 C thermal temperature for 10 min ceased germination (Thompson et al. 1997). Effective germination reduction of buried annual bluegrass (Poa annua L.) seeds was achieved with a total of 66 h above 45 C (Peachey et al. 2001).

Regardless of the method and weed species, the two primary interactive factors that influence thermal weed control are temperature achieved and exposure time. In general, higher temperatures require shorter duration and lower temperatures require a longer duration. To achieve 100% mortality of barnyardgrass [Echinochloa crus-galli (L.) Beauv.], exposure time varied among treatment temperatures. A short duration (0.17 h) required 70 C for 100% barnyardgrass mortality. When decreasing thermal temperature to 46 C, 16 h exposure period was required to achieve the same result. Extended exposure periods (56 h) of 50 C were required to achieve 100% common purslane (Portulaca oleracea L.) mortality (Dahlquist et al. 2007). When common purslane seeds were placed in moist soil, the thermal heat exposure period increased to 7 d for effective germination reduction (Egley 1990). When decreasing thermal exposure to velvetleaf (Abutilon theophrasti Medik.) seeds by 9 h, a 10 C increase was required to inhibit germination (Horowitz and Taylorson 1983). Similar results have been observed in common lambsquarters (Chenopodium album L.) and Indian mustard [Brassica juncea (L.) Czern.] (Hopkins 1936; Thompson et al. 1997). These studies indicate that one important effect of reduced thermal heat exposure time is an increase in the temperature required to achieve substantial decreases in seed germination.

The major limitation to thermal weed seed control methods is low temperature applications. Thermal weed control methods that are of lower heat intensity require longer exposure times for adequate weed germination reduction. Longer exposure times decrease the potential treated acreage. Thermal dry heat applications can only treat 1 to 2 ha d^{-1} (Williams 1999). Mobile steaming units can only be applied at 40 to 100 h ha⁻¹ (Bond et al. 2007). Other low intensity–longer exposure time applications, such as solarization, can require up to a 6-wk application period (Horowitz et al. 1983). Commonly, these thermal weed control methods achieve extended heat exposure periods.

We are currently testing thermal weed control methods utilizing a PL8750 flame sanitizer[®] (Red Dragon, Model PL8750 Poultry House Flame Sanitizer, Flame Engineering, Inc. P.O. Box 577, LaCrosse, Kansas 67548) (Figure 1) for stale-seed-bed preparation prior to turfgrass establishment



Figure 1. PL8750 flame sanitizer demonstrating short thermal heat exposure by flame.

(Hoyle et al. 2011a). This application method utilizes intense thermal heat from six, 3.3×10^5 W h (watt hours) torches, individually reaching approximately 1,121 C flame temperature. This method applies high intensity heat for a much shorter time duration (≤ 20 s), depending on speed. The manufacturer-recommended operating speed for this unit is 0.8 kilometers per hour (kph) and a recommended fuel pressure of 345 kPa. Therefore, the purpose of this study was to investigate increased thermal temperatures at short exposure periods on seed mortality of large crabgrass, cock's-comb kyllinga, and Virginia buttonweed.

Materials and Methods

Cock's-comb kyllinga (Cyperaceae), a tufted summer annual and Virginia buttonweed (Rubiaceae), a mat-forming spreading perennial herb, were harvested at Auburn University's Turfgrass Research Unit (32°34'38.57"N, 85°29'59.76"W) located in Auburn, AL. Mature cock'scomb kyllinga plants were harvested with a rotary mower in summer 2010. Clippings and seeds were allowed to air-dry. Seeds were separated from clippings by sieving. Virginia buttonweed fruits were collected in December 2010 at same location utilizing a 9.5 L vacuum (Shop-Vac, 9.5 L/2.5 horsepower wet/dry vacuum, Lowes, Cornelius, NC, 28031). Dried fruits were removed from the soil surface surrounding Virginia buttonweed populations. Sieving separated fruits from other materials. Virginia buttonweed seeds are normally dispersed with fruit tissue surrounding the seeds and germinate within the intact pericarp in natural environments. Initial experiments showed no influence on seed germination of separating seeds from pericarps; therefore, seed and pericarp were left intact. Harvested seeds were mixed to ensure a homogenous mixture. Large crabgrass seed was purchased (Elstel Farm and Seeds, 2640 Springdale Road, Ardmore, OK 73401). All seeds were stored at 10 C and 50% relative humidity (RH) prior to use.

Laboratory experiments were conducted at Auburn University, Auburn, AL to determine the germination response of the common turfgrass weeds to thermal heat. Thirty seeds or fruits were counted, separated, and stored in a cooler (10 C) before heat treatments were applied. A two-by-seven

completely randomized design with a factorial treatment arrangement was utilized with temperatures of 60, 80, 100, 120, 160, 200, and 250 C and exposure times of 5 and 20 s. The 5 s exposure time corresponds to the thermal heat exposure of the PL8750 flame sanitizer[®] at the recommended manufacturer operating speed of 0.8 kph. The 20 s exposure treatment was chosen for comparison purposes.

Two experimental runs for each weed species were conducted with four replications of each combination treatment and included a nonheat treatment. The weed species tested were large crabgrass, Virginia buttonweed, and cock'scomb kyllinga. The thermal heating units utilized convection ovens (Euro-Pro Kitchen Convection Toaster Oven, TO36, Euro-Pro Operating LLC, Boston, MA 02459). One convention oven was utilized for seed treatment and is denoted as "treatment oven" (TO). A separate convection oven was utilized to heat ceramic crucibles (Low form crucible, 7.5 diam. 00A 51-K, Coors crucibles, VWR International, Randor, PA) and is denoted as "crucible oven" (CO). A probe attached to an infrared thermometer (IR2-S Infrared Thermometer with Probe, Turf-Tec International, Tallahassee, FL 32303) was inserted through the top of each convection oven to monitor oven temperature. Before applying the treatments, the TO and CO were set to the specified temperature, and the targeted temperature confirmed using an infrared thermometer. Once the convection ovens attained the appropriate temperature, four crucibles were inserted into the CO. After 3 min, a crucible was removed from CO. The target crucible temperature was confirmed using a separate infrared thermometer. Thirty seeds or fruits of a single weed species were placed in the crucible and then inserted in the TO for the 5 or 20 s exposure times. This methodology provided conduction and convection thermal heat transfer simultaneously. Following heat treatment, seeds were immediately placed in 9-cm-diam Petri dishes (Petri Dish, VWR International) containing two pieces of #2 filter paper (Filterpaper, VWR International), and moistened with 6 ml of deionized water. Petri dishes were sealed with parafilm (Parafilm, Pechiney Plastic Packaging, Chicago, IL 60623) and placed in a growth chamber (Growth Chamber, Adaptis A1000PG, Conviron, 590 Berry St., Winnipeg, Manitoba, Canada). Environmental conditions in the growth chamber were 50% RH, alternating (day/night) photoperiod and temperature of 16 and 8 h and 30 and 20 C, respectively. Light was applied from fluorescent lamps (650 μ mol m⁻² s⁻¹, 39-watt T5HO/840 fluorescent lamps, Conviron). Counts were conducted every 7 d until germination ceased, approximately 6 wk after the start of the germination trial. A seed was considered germinated if the radicle was visible. Germinated seeds were counted and removed. Counts were totaled at the conclusion of the study. Seed viability of nongerminated seeds was determined using the tetrazolium test (Moore 1985). Staining patterns from the tetrazolium test were difficult to interpret because of the inconsistency of the lactophenol clearing solution; therefore, no viability data are presented. However, Dahlquist et al. (2007) reported the presence of nonviable, ungerminated seeds after exposure to thermal heat treatments. Germination counts were converted to mortality percentages relative to nontreated seeds.

Mortality data of large crabgrass, Virginia buttonweed, and cock's-comb kyllinga as influenced by exposure time and temperature were analyzed using SAS (SAS 2011). Data were combined for analyses across experimental runs. For all weed species, increasing thermal temperatures allowed for nonlinear regression analysis. Mortality for each exposure time and temperature combination was calculated by each replication within each experimental run from total relative germination counts. Percent mortality of each weed species and exposure time was regressed against temperature using SigmaPlot (Sigmaplot 11.2[®] for Windows[®]. SPSS, Inc., 444 North Michigan Ave., Chicago, IL 60611). Initial analysis resulted in no hormesis effect (Brain and Cousens 1988). The threeparameter sigmoid regression model (Myers 1986) proved useful in estimating the lethal thermal death temperature for various weeds at different exposure times. Differences in percent mortality of weed species due to temperature treatment and exposure time were determined using the following sigmoid regression model shown in Equation 1:

$$y = a / \left\{ 1 + e^{[-(x - M_{50})/b]} \right\}$$
[1]

where y is the response (percent mortality) at temperature x, M_{50} (temperature to achieve 50% mortality), *a* is the upper limit (% mortality), and b is the slope (at M_{50}). Lack-of-fit tests were performed in accordance with Seefeldt et al. (1995), Melander and Jrgensen (2005), and Melander and Kristensen (2011) to clarify whether Equation 1 or the full ANOVA model best described the data. Models were also compared on the basis of F-tests (Melander and Jrgensen 2005; Melander and Kristensen 2011). A full model was set up in which a, b, and M_{50} parameters were dependent on exposure time (5 and 20 s) and temperature (60, 80, 100, 120, 160, 200, and 250 C). As outlined by Melander and Kristensen (2011), the model was successively reduced and F-tests were used to identify significance between models according to the sum of squares reduction tests described by Brown and Rothery (1993). For presentation purposes, means with error bars based on the standard error (P = 0.05) as determined by SAS (2011) were graphed in SigmaPlot, which had been generated previously.

Results and Discussion

Significant temperature (F = 621.01, P = < 0.001) and exposure time (F = 121.18, P = < 0.001) main effects, as well as an interaction between temperature and exposure time (F =73.69, P = < 0.001) were observed for large crabgrass. Interaction allowed for nonlinear regression model selection. Sigmoid regression curves (Equation 1) explained large crabgrass mortality by temperature for each exposure period (Adjusted $R^2 \ge 0.97$ for 5 and 20 s models). The equation described data as well as the full ANOVA model. Successive model reduction showed that exposure period and temperature influenced the estimation of M_{50} values. The model ultimately resulted in M_{50} estimates representing actual temperature to achieve 50% large crabgrass mortality, as was also the case for cock's-comb kyllinga and Virginia buttonweed. Cock's-comb kyllinga required the lowest temperature and large crabgrass required the highest temperature to achieve 50% mortality at the 5 s exposure period. Large crabgrass required the lowest temperature and Virginia buttonweed required the highest temperature to achieve 50% mortality at the 20 s exposure period.

Parallel herbicide dose-response models could indicate that herbicides are acting on the same site of action if comparing herbicide-resistant weed biotypes (Streibig et al. 1993). When comparing thermal temperature exposure times to weed seed mortality, one would assume that thermal heat is acting upon the same site. High thermal heat affects organisms differently by changes in membrane properties (Hendricks and Taylorson 1976, 1979), denaturation of proteins, change in viscosity of membrane lipids (Christiansen 1978; Esser and Souza 1974; Labouriau 1977; Levitt 1969; Volger and Santarius 1981), and heat shock proteins (Coca et al. 1994; Medina and Cardemil 1993). Low temperatures might not alter the seed properties enough to increase mortality or affect different physiological aspects of the seeds. At the 5 and 20 s exposure times, lower temperatures simply affect properties of Virginia buttonweed seed differently. Further investigation of Virginia buttonweed seed morphology and thermal heat tolerance could potentially provide insight into physiological changes that might be taking place.

Weed Seed Mortality. Mortality increased with increasing temperature but varied by weed species and exposure time. Large crabgrass, Virginia buttonweed, and cock's-comb kyllinga achieved maximum mortality of 100, 97, and 100%, respectively. Virginia buttonweed mortality was 100% at 200 and 150 \bar{C} for 5 and 20 s exposure times (Figure 2), although the sigmoid regression model estimated the maximum mortality at 97%. Sigmoid regression models for each weed species by exposure time were utilized in determining 50% mortality of large crabgrass, cock's-comb kyllinga, and Virginia buttonweed (Table 1). Increased exposure time reduced the thermal temperature required to achieve 50% mortality in large crabgrass and cock's-comb kyllinga. Increasing thermal exposure time from 5 to 20 s reduced the thermal temperature by 21 C to achieve 50% mortality for large crabgrass and by 10 C for cock's-comb kyllinga. Virginia buttonweed mortality was 50% at the same 5 and 20 s exposure thermal temperature (99 C). Not removing pericarps likely increased Virginia buttonweed heat tolerance; however, intact pericarps are more realistic of field germination conditions.

The damaging effects of high temperature could be related to changes in membrane properties (Hendricks and Taylorson 1976, 1979). Egley (1990) reported that seed coat-imposed dormancy could be broken with 50 to 60 C temperatures, thus enhancing seed germination. The stimulation of seed germination can be induced by heat when hard seed coats are broken, which facilitates imbition and radicle growth (Herranz et al. 1998). Antagonistically lower thermal temperature with slightly longer exposure time (20 s) could possibly denature Virginia buttonweed pericarps and stimulate germination. Insufficient heat exposure duration at 5 s did not break dormancy or stimulate germination. Eventually, increased mortality was observed at increased thermal temperatures at the 5 s exposure period.



Figure 2. Sigmoid regression models as determined by Equation 1 for large crabgrass, cock's-comb kyllinga, and Virginia buttonweed percent mortality at 5 and 20 s exposure times. Abbreviations; s, second; y, percent mortality; x, temperature (C); Adj, adjusted.

Egley (1990) reported that longer exposure periods at lower temperatures were more destructive for weed seed germination than shorter periods at higher temperatures. This conclusion is not comparable with the present data because of substantial differences in exposure periods between the Egley (1990) study and this experiment. Egley (1990) utilized exposure periods ranging from 0.25 to 7 d, whereas exposure periods in this study were 5 and 20 s. Similar to data obtained

Weed species	Exposure time (s)	a ^b	Ь	M ₅₀	Adj R ²
Large crabgrass	5	100.08 ± 0.91	3.03 ± 2.27	103.52 ± 2.69	0.97
	20	100.08 ± 0.91	3.03 ± 2.27	82.75 ± 2.11	0.98
Virginia buttonweed	5	96.53 ± 1.74	15.53 ± 1.93	98.68 ± 1.32	0.90
	20	96.53 ± 1.74	9.94 ± 1.46	98.68 ± 1.32	0.91
Cock's-comb kyllinga	5	101.08 ± 1.85	7.51 ± 0.97	97.08 ± 1.47	0.84
	20	101.08 ± 1.85	7.51 ± 0.97	87.49 ± 1.61	0.90

Table 1. Parameter estimates (\pm standard errors) from fitting Equation 1 to data sets for large crabgrass, Virginia buttonweed, and cock's-comb kyllinga mortality due to exposure temperature.^a

^a Sigmoid regression model defined by Equation 1.

^b Abbreviations: *a*, maximum mortality: *b*, slope; *M*₅₀, temperature to achieve 50% mortality; *Adj*, adjusted.

by Thompson et al. (1997), all three target weed species reached thermal death points regardless of thermal heat exposure time. The maximum temperature required to prevent germination is more important than the exposure time of heating, although exposure time can influence efficacy of thermal heat treatments (Thompson et al. 1997). However, black nightshade (Solanum nigrum L.), hairy galinsoga (Galinsoga quadriradiata Cav.), green foxtail [Setaria viridis (L.) Beauv.], common purslane, and redroot pigweed seed germination was severely affected by short exposure to thermal temperatures (Vidotto et al. 2011). Temperature and sprouting ability of quackgrass [Elymus repens (L.) Gould] rhizome buds was inversely related to exposure time (Melander et al. 2011). Melander and Kristensen (2011) found no influence of heat duration when soil steaming for 3 to 12 s. Although exposure times used in our experiment and by Melander and Kristensen (2011) are similar, differences in temperature to achieve 50% weed mortality of large crabgrass and cock's-comb kyllinga at 5 and 20 s durations were found in our study.

Implications for Weed Control. Large crabgrass, cock'scomb kyllinga, and Virginia buttonweed seeds subjected to a thermal heat of 99 C for 20 s resulted in 50% mortality. Increasing thermal heat by 4 C (103 C) and reducing exposure time by 15 s can provide similar mortality as a 20 s exposure period. Thermal weed control measures must achieve these temperatures for short exposure times at the seed surface interface to achieve the thermal death point.

Thermal heat at reported temperatures does not only influence weed seed mortality but also can cause microbial disruptions at any temperature greater than 60 C (DeBano et al. 1998). Also, when intense heat (300 C) is applied to any soil surface, negative effects can occur to the soil (Certini 2005). Long- or short-term thermal heat influences on soil (DeBano et al. 1998) can affect organic carbon (Giovannini et al. 1988), soil permeability (Imeson et al. 1992), pH (Arocena and Opio 2003), bulk density (Giovannini et al. 1988), and available nutrients (Fisher and Binkley 2000). Although soils are negatively impacted at approximately 300 C, the targeted temperature for effective thermal weed seed population reduction of species used in this study is 103 C, but the thermal heat must be present at the seed location.

Thermal heat must transfer through soil to depths where seeds are located at the sufficient temperature and time to be effective. Heat movement through the soil depends on volumetric proportions of solid, liquid, and gas phases, arrangement of solid particles, and interfacial contact between the solid and liquid (Jury and Horton 2004). Thermal conductivity decreases with decreasing particle size (Patten 1909), increases with bulk density (van Rooyen and Winkerton 1959), and increases with water content (van Duin 1963; van Rooyen and Winkerton 1959). Experiments have been conducted to determine maximum emergence depths for large crabgrass, cock's-comb kyllinga, and Virginia buttonweed in various soil textures (Hoyle et al. 2011b) along with heat movement through soil (Ochsner et al. 2001; van Duin 1963).

Seed moisture also influences seed mortality (Riemens 2003) and affects seed susceptibility to heat (Horowitz and Taylorson 1983). Previous research concluded that dry seeds of barley (Hordeum vulgare L.) withstood heat exceeding 100 C (Couture and Sutton 1980). Experiments in our study were conducted on nonimbibed seeds. Therefore, heat effects on seeds can be enhanced by high seed moisture content (Egley 1990; Melander and Jrgensen 2005). Similar to our study, previous research has shown that dry seeds are less susceptible to heat exposure, and increased temperatures are needed to attain the same mortality levels (Bloemhard et al. 1992; Egley 1990; Melander and Jrgensen 2005; van Loenen et al. 2003). From a practical perspective, mean higher soil temperatures might be required to kill weed seeds in the upper soil layer where long, dry periods precede thermal treatment (Melander and Jrgensen 2005).

Many factors influence the efficacy of thermal weed control methods. Better knowledge of the exposure times and thermal temperatures needed to achieve thermal weed seed death is critical for the development and implementation of efficacious short-exposure thermal weed control methods.

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